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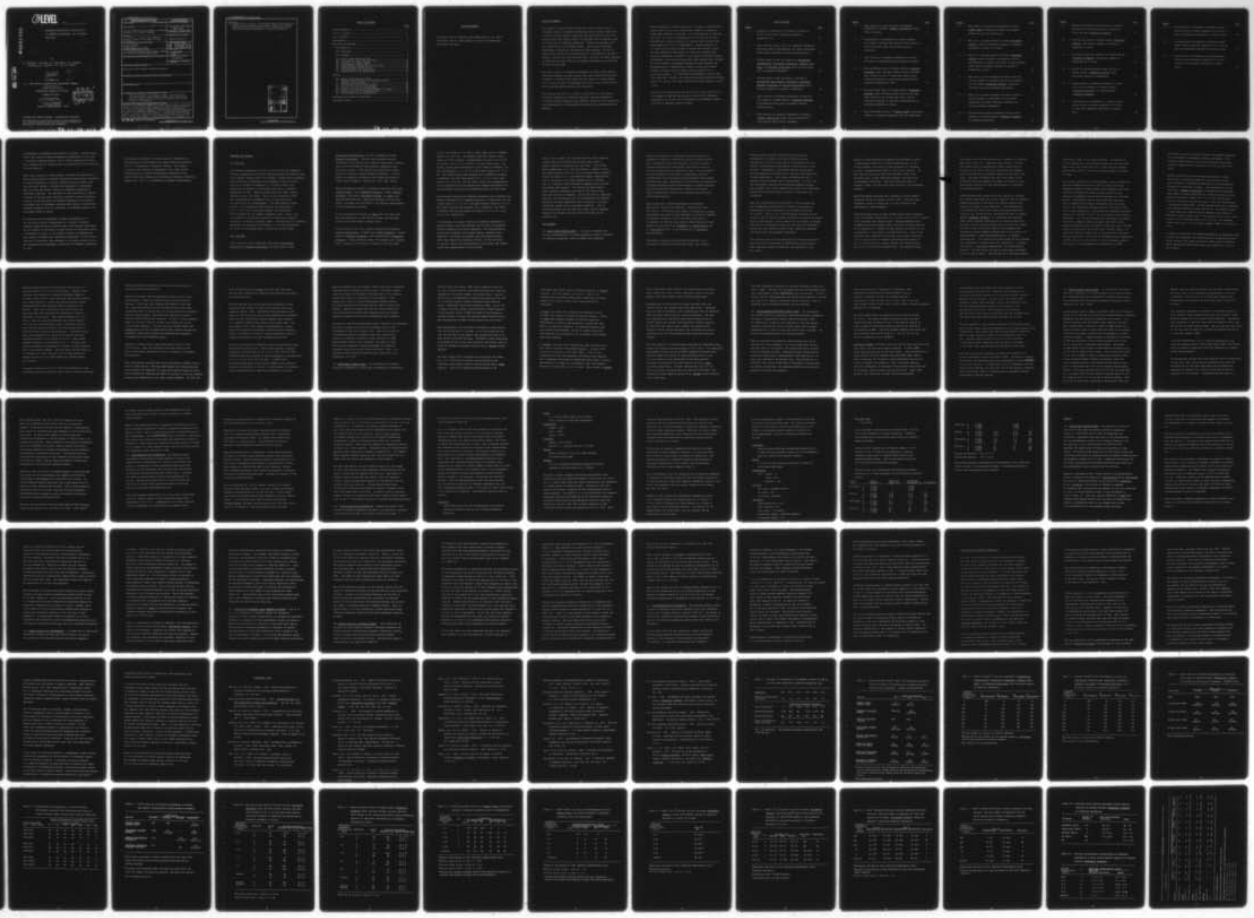

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The toxicity of elemental phosphorus to a wide variety of aquatic organisms was studied. Results of static acute toxicity tests indicate that the acute LC50 values varied over several orders of magnitude with an observed range of 6 - >560 µg/l of elemental phosphorus. Chronic exposure of fathead minnows for 150 days to concentrations ranging from 1.5 - 3.4 µg/l significantly reduced growth. In addition, survival was affected in concentrations ≥1.5 µg/l, while eggs spawned by fish exposed to the low concentrations of 0.71 and 0.40 µg/l failed to hatch when incubated. Although bioaccumulation of elemental

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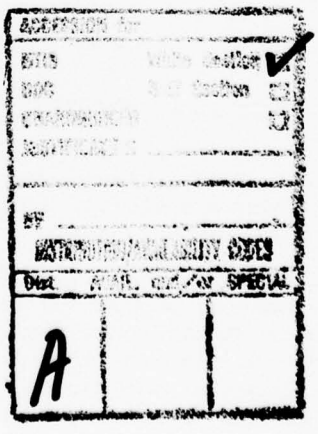
greater than 560 microgram/l

Equal to or greater than 1.5 microgram/l

(continued)

20. phosphorus was low (ca 200X), the cumulative mortality pattern observed suggests continuing damage to target organs. Based on the available data and the use of a safety factor of 0.1, an ambient water quality criterion for elemental phosphorus of 0.04 µg/l is recommended.

microgram/l



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EXECUTIVE SUMMARY

The results of static acute toxicity tests with a wide variety of freshwater aquatic organisms representing several trophic forms under a variety of water quality conditions indicates that the acute LC50 values varied over several orders of magnitude with an observed range of 6->560 ug/l of elemental phosphorus. Fishes were generally the most sensitive of the organisms tested. During static bioassays temperature, alkaline pH, and moderate water hardness (100 mg/l as $CaCO_3$) had no affect on the acute toxicity of phosphorus to bluegill. However, acid pH (6.0) and very hard water (250 mg/l as $CaCO_3$) appeared to decrease the acute toxicity of elemental phosphorus to bluegill by an order of magnitude.

The acute toxicity of elemental phosphorus to fishes during dynamic continuous exposure was much greater than that observed during static bioassays. Incipient LC50 values for two species of fishes tested were at least an order of magnitude less (0.6-4.2 ug/l) than 96 hour LC50 values determined during static bioassays.

The presence initially of a nominal concentration of 500 ug/kg of elemental phosphorus in sediment completely inhibited production of eggs by midges inhabiting that sediment during the first generation and subsequently by reintroduced midges during the second generation exposure.

Chronic exposure of fathead minnows for 150 days to concentrations ranging from 1.5-3.4 ug/l significantly reduced the growth of fishes when compared to controls. At the termination of exposure (day 241), all fish in concentrations of 6.0 ug/l and 3.4 ug/l had died and a further reduction in survival had occurred among fish exposed to 1.5 ug/l of phosphorus. Although exposure to 0.71 and 0.40 ug/l had no effect on growth and survival, nearly all eggs spawned by fish exposed to those low concentrations failed to hatch when incubated in the respective exposure aquaria. Furthermore, even when egg groups from these aquaria were incubated in control aquaria, percentage hatchability was extremely low. Bioaccumulation of phosphorus by channel catfish and fathead minnows, although initially rapid, did not appear to be a continuing process. The calculated bioaccumulation factors were less than 200X. However, the cumulative mortality pattern observed during the various fish tests suggests continuing damage to target organs.

Based on the available data and the use of a safety factor of 0.1 we recommend an ambient water quality criterion for elemental phosphorus of 0.04 ug/l for the protection of freshwater aquatic life with an adequate margin of safety.

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INTRODUCTION

Elemental white phosphorus (P_4) is known to occur in discharges from the Pine Bluff Arsenal in Pine Bluff, Arkansas. Shook (1972) reported mean measured P_4 concentration of 32 mg/l in the discharge from the settling pond at Pine Bluff Arsenal. This discharge flows in a ditch to Yellow Lake. Although much of the P_4 apparently settles out in the ditch, the concentration of P_4 in the water entering Yellow Lake is 1-3 mg/l (Envirotech Systems Inc., 1971). Furthermore these authors reported that much of the settled out P_4 is resuspended and washed into Yellow Lake after heavy rains. Concentrations of P_4 in the water of Yellow Lake were reported as a mean of 0.22 mg/l (range, 0-2.6 mg/l), and a range of 0.1-3.0 mg/l in the sediments (Rosenblatt et al., 1973). Pearson et al. (1976) have reported concentrations of P_4 in the water and sediments of Yellow Lake ranging from 0.02-40.4 ug/l and 0.02-43.4 ug/kg, respectively. It was further reported that high water on the Arkansas River is sufficient to cause river water to back up through the outlet of Yellow Lake as often as 2-3 times a year (Rosenblatt et al., 1973). A pessimistic analysis has been constructed which results in estimated short-term concentrations of P_4 in the Arkansas River, and in the Mississippi River downstream of the Arkansas River of 0.007 mg/l and 0.002 mg/l, respectively (Rosenblatt et al., 1973).

One of the earliest investigations of the toxicity of elemental phosphorus to fish was that of Isom (1960) using bluegill sunfish as test organisms. He reported that phosphorus in the colloidal form was toxic in concentrations ranging from 25-100 parts per

billion (ug/l). However, in the light of present knowledge of the stability of solubility of elemental phosphorus in both freshwater and marine aqueous solution (Bullock & Newlands, 1969) it is probable that the effective concentrations were much lower than those reported.

In 1970, following the pollution of Placentia Bay in Newfoundland by yellow phosphorus (Jangaard, 1972) several workers began investigations of the effects of elemental phosphorus on aquatic organisms. Zitko et al. (1970) reported that in the herring, salmon, and lobster the toxic effect of phosphorus is irreversible and probably cumulative. They reported incipient lethal levels of yellow phosphorus for lobster, salmon, and beach fleas were 40 ug/l, 18 ug/l and 3-4 mg/l, respectively. However, they also reported no clear indication of an incipient lethal level was found for herring, with acute toxicity observed at concentrations as low as 2.5 ug/l. Fletcher et al. (1970) reported yellow phosphorus was lethal to seawater maintained brook trout and smelt as low as 0.5 ug/l. Much of the previous work concerning the uptake of phosphorus by fish was concentrated on a marine species of cod (Gadus morhua). This species accumulated phosphorus rapidly with particularly high concentrations reported in liver (Dyer et al. 1970, Fletcher 1974, Maddock and Taylor 1975). However, cod may not be a representative species due to the high lipid content and liver oils which are characteristic of them. Concentrations of phosphorus in fish tissues are apparently directly related to the concentration in water, and are eliminated rapidly when fish are removed from exposure (Fletcher, 1974). Information concerning the accumulation

of phosphorus by freshwater fish species is limited. Pearson et al. (1976) have recently reported phosphorus accumulation in the liver of laboratory exposed bluegill, and in catfish sampled from Yellow Lake, Arkansas during investigations of contamination from a munitions filling operation.

Since acute toxicity to marine aquatic organisms was observed at or below concentrations of elemental phosphorus conservatively estimated to occur periodically in natural streams receiving effluent from the Pine Bluff Arsenal, a program was undertaken to investigate both the acute and chronic toxicity of elemental phosphorus to freshwater organisms. The objective of this program was to provide a portion of the data base required to perform a hazard evaluation relative to the occurrence of elemental phosphorus in the aquatic environment and to recommend a proposed water quality criterion for elemental phosphorus for the protection of freshwater aquatic life with ample margin of safety.

The specific efforts undertaken included investigations of: (a) the acute toxicity of phosphorus to a variety of aquatic organisms under static and flowing water (flow-through) conditions; (b) the effects of variations in water quality on the acute toxicity of phosphorus to fish; (c) the chronic toxicity of phosphorus to both aquatic vertebrate and invertebrate organisms; (d) the chronic toxicity of phosphorus laden sediment to the benthic midge; and (e) the accumulation and subsequent elimination of elemental phosphorus by fish.

The studies to evaluate the acute toxicity of phosphorus to phytoplankton were performed at the Marine Research Laboratory of E G & G, Bionomics in Pensacola, Florida. The studies to evaluate the toxicity of phosphorus to all other aquatic organisms and all chemical analyses were conducted at the Aquatic Toxicology Laboratory and Analytical Chemistry Laboratory, respectively, of E G & G, Bionomics in Wareham, Massachusetts.

MATERIALS AND METHODS

Test Material

The elemental phosphorus utilized in these studies was elemental (yellow) phosphorus (100% active) and was obtained from Fisher Scientific Company (Catalog #P104, Lot #767980). During static bioassays, stock solutions of phosphorus were prepared by placing a small quantity of elemental phosphorus in 9 liters of water, and subjecting the solution to continuous stirring at a constant temperature of 43°C. These solutions were held under constant pressure (4 psi) with argon. Prior to the initiation of each test concentration of phosphorus (P_4) in stock solutions was measured by gas chromatography. The mean measured concentration of the phosphorus stock solution was determined to be 2.6 (± 0.1) mg/l, which was assumed to be saturation. Stock solutions for all dynamic bioassays, acute, chronic, and bioaccumulation studies were prepared by dissolving elemental phosphorus in dimethyl sulfoxide (DMSO). Concentrations of elemental phosphorus are reported as micrograms (ug) of active ingredient per liter (l) of diluent water, or parts per billion (ppb).

Test Organisms

Algae tested were the cyanophytes (blue-green) Microcystis aeruginosa and Anabeana flos-aquae; the chlorophyte (green)

Selenastrum capricornutum; and the chrysophyte (diatom) Navicula pelliculosa. Cultures were obtained from the collection at the University of Indiana, Bloomington, Indiana, and the Pacific Northwest Water Quality Laboratory (EPA, Corvallis, Oregon). Each species was maintained in stock cultures at Bionomics Marine Research Laboratory. Culture medium was prepared according to the formula described in "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971a).

Macroinvertebrates exposed to phosphorus were the water flea (Daphnia magna), scud (Gammarus fasciatus), sowbug (Asellus militaris), and midge (Chironomus tentans). D. magna were acquired from Bionomics' laboratory cultures, and the scud, sowbug, and midge were collected in the Wareham, Massachusetts area by Bionomics' personnel.

At the initiation of testing, D. magna were 0-24 hours old, scud and sowbug were in the juvenile stage; and the midge larvae were in the second or third instar.

Fish utilized in the acute, static bioassays were bluegill (Lepomis macrochirus), rainbow trout (Salmo gairdneri), channel catfish (Ictalurus punctatus), and fathead minnow (Pimephales promelas). Unless otherwise noted, the bluegill were acquired from a commercial fish farmer in Nebraska, and had a mean

(± S.D.) wet weight of 1.0 (±0.3) g and a mean (± S.D.) standard length of 35 (± 6) mm. The rainbow trout were acquired from a commercial trout producer in Massachusetts and had a mean weight and length of 0.9 (± 0.3) g and 43 (± 4) mm, respectively. The channel catfish were obtained from a fish farmer in Arkansas, and had a mean weight of 1.2 (± 0.5) g and a mean length of 47 (± 11) mm. The fathead minnow were obtained from a commercial producer in Arkansas, and had a mean weight of 0.1 (± 0.4) g and a mean length of 43 (± 8) mm. For all tests, thirty fish representative of test populations of each species were weighed and measured for the calculation of means and standard deviations for each group.

Flow-through bioassays with elemental phosphorus were conducted with bluegill obtained from a commercial hatchery in Connecticut and had a mean weight of 1.7 (± 0.5) g and mean standard length of 40 (± 3) mm; channel catfish were acquired from a commercial fish farmer in Missouri and had mean weight and length of 2.1 (± 0.2) g and 43 (± 3) mm, respectively.

To investigate the chronic toxicity of elemental phosphorus to fathead minnows, eggs were obtained from the EPA, Environmental Research Laboratory in Duluth, Minnesota. The bioaccumulation studies were conducted with channel catfish, obtained from a commercial fish farmer in Arkansas and with fathead minnows, which were reared from laboratory stocks. At the initiation of testing, catfish were approximately 5g wet weight and fathead minnow were approximately 0.3g wet weight.

Prior to use in tests, all fish were held in 1700-l concrete raceways which were coated with an epoxy resin paint to prevent leaching of materials into the water. Flow of well water (temperature, 20 $\pm$ 1.0°C for bluegill, channel catfish, and fathead minnow, and 14 $\pm$ 1.0°C for rainbow trout; hardness, 35 mg/l as $CaCO_3$; pH 7.1, and dissolved oxygen concentration, >60% of saturation) into these raceways was at a minimum rate of 4 l/minute, which provided an adequate water turnover for holding these species. The fishes were maintained in these laboratory hatchery facilities for at least thirty days prior to use in bioassays. During this period, cumulative mortality for each species was <2%; no mortality was observed during the 48 hours immediately prior to testing, and these fishes were judged to be in excellent condition. Fish of each species were from the same year class, and the standard length of the longest fish was no more than twice of the shortest fish.

Test Methods

(A) STATIC ACUTE TOXICITY TESTS - In order to evaluate the relative susceptibility of a broad spectrum of aquatic organisms to elemental phosphorus, static bioassays were conducted.

During all bioassays to investigate the acute toxicity of phosphorus to aquatic organisms two series of concentrations were established within each bioassay, a series of range-finding concentrations (preliminary test) and a series of definitive concentrations (definitive test). The preliminary test was conducted to determine an approximate range of concentrations for evaluating the dose-response relationship. The definitive test, consisting of at least five concentrations, evaluated the dose-response relationship to a degree allowing the median effective concentration (EC50) or the median lethal concentration (LC50) to be calculated from the data with optimum accuracy.

Algal assays were conducted according to the method described in "Algal Assay Procedure: Bottle Test" (U.S. EPA, 1971a). To determine the effects of phosphorus on algae, measurements were made of the chlorophyll *a* content of exposed and control cultures of each of the four test species. In addition, to confirm these results, determinations of cell numbers for cultures of *M. aeruginosa*, *S. capricornutum* and *N. pelliculosa* and of optical density for *A. flos-aquae* were performed.

Chlorophyll *a* analyses were conducted according to the procedures of Strickland and Parsons (1972) and involved

filtering algal cultures from test medium, extracting chlorophyll by treatment of algal cells with acetone, determining extinction values with a spectrophotometer and finally, calculating the chlorophyll a concentration in the solution. Chlorophyll a and optical density measurements (at 680 nanometers) were made with a Bausch & Lomb Spectronic 20 spectrophotometer. Cell counts were performed with a compound light microscope and a hemacytometer. In each case, the measurements obtained from triplicate exposed cultures were averaged, the results compared with those from triplicated controls and a percent effect (relative to controls) was calculated.

Each test concentration was converted to its logarithm and the corresponding percent effect (change in chlorophyll a concentration, optical density or cell number) converted to a probit. The 24-, 48-, and 96-h median effective concentrations, EC50's (concentrations effective in changing the chlorophyll a concentration, optical density or cell number of exposed algae by 50% as compared to controls) and their 95% confidence limits were then estimated from a linear regression equation calculated with a programmable calculator.

Test methods used for static bioassays with macroinvertebrates and fishes were as described in "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (U.S. EPA, 1975).

Results of macroinvertebrate bioassays are expressed as EC50's (concentrations effective in causing immobilization of 50% of test animals) and results of fish bioassays are expressed as LC50's (concentrations lethal to 50% of test animals). The EC50 and LC50 values and their 95% confidence limits were estimated from a linear regression equation calculated with a programmable calculator. Data from replicates were not averaged, rather, all data pairs were utilized in the regression analysis.

Macroinvertebrate bioassays were conducted in 250-ml beakers containing 200 ml of solution at 20 $\pm$ 1.0°C. Aged well water (hardness, 35 mg/l as $CaCO_3$; pH, 7.1) was utilized in the performance of these bioassays.

Dissolved oxygen values in test vessels during static bioassays with invertebrates ranged from 8.0 to 8.2 mg/l (87% to 90% of saturation) throughout the testing period. Macroinvertebrates were introduced into test beakers within 30 minutes following addition of the phosphorus; 15 animals of each species were tested at each concentration (3 replicates, 5 animals/replicate). Static fish bioassays were conducted in 19.6-liter glass vessels containing 15-l of solution held in constant temperature water baths at 20 $\pm$ 1.0°C for bluegill, channel catfish, and fathead minnow, and at 10 $\pm$ 1.0°C for the rainbow trout. The standard diluent

(well water) for the fish species had a hardness of 35 mg/l as $CaCO_3$ and a pH of 7.1. Dissolved oxygen values in various test vessels during bioassays with fishes ranged from 9.0 initially to 4.0 mg/l (99%-43% of saturation for warmwater species and 80%-35% for coldwater species) at the end of the tests. Fish were introduced into each test vessel within 30 minutes after the compound was added; 30 animals of each species were utilized for each concentration (3 replicates, 10 animals/replicate).

Fathead minnows were chosen as the test species to evaluate the relative susceptibility of life stages of fish to elemental phosphorus because of the ability to readily procure their various life stages in the laboratory. The susceptibility of selected life stages (egg, 1-hour old newly-hatched fry, 7-day old fry, 30-day old fry, and 60-day old fry) of fathead minnow (Pimephales promelas) to phosphorus was evaluated under static bioassay conditions for a 144-hour period with the eggs, and for a 96-hour period with all other life stages. The egg, 1-hour old fry and 7-day old fry bioassays were conducted in 250-ml beakers containing 200 ml of solution (10 animals/beaker, 3 replicates/concentration, 30 animals/concentration). The 30-day old fry and 60-day old fry bioassays were conducted in 1-gallon glass jars containing 3 l solution (10 fry/jar, 3 replicates/concentration, 30 animals/concentration). The LC50 values for the egg tests were calculated at 24, 48 and 144 hours. The time period of 144 hours allowed

100% hatch of eggs in all control beakers. In addition to percent mortalities, percent hatch of eggs was also observed. These tests were conducted at 25 $\pm$ 1.0°C, and the standard diluent had a pH of 7.1 and total hardness (EDTA) of 35 mg/l as $CaCO_3$.

Due to their sensitivity to the chemicals, their availability, and their expected presence in most of those areas where phosphorus might be found, bluegill were selected as the test species to evaluate the effect of water quality on the toxicity of phosphorus during static bioassays for a 96-hour period. The bluegill used in these tests were obtained from a commercial fish farmer in Nebraska, and had a mean ($\pm$ S.D.) wet weight and standard length of 0.9 ($\pm$0.2) g and 33 ($\pm$ 5) mm, respectively. Bioassays were conducted utilizing bluegill to determine the 24-, 48-, and 96-hour LC50 values of phosphorus: (a) at three temperatures representing the lower end (15°C), midpoint (20°C), and upper end (25°C) of the normal temperature range for bluegill using soft water (35 mg/l $CaCO_3$) at neutral pH; (b) in soft water (35 mg/l $CaCO_3$), in hard water (100 mg/l $CaCO_3$) and in very hard water (250 mg/l $CaCO_3$) using water of pH 7.0 at the recommended test temperature of 20°C; and (c) at pH's of 6.0, 7.0 and 8.0 using standard soft water at the recommended test temperature of 20°C.

The diluent for each of these conditions was prepared according to the procedures recommended by Marking and Dawson (1973). Dissolved oxygen values in various test vessels during these bioassays ranged from 9.0 initially to 4.2 at the end of the tests.

General availability and their expected presence in those areas where the phosphorus might be found resulted in the use of bluegill to evaluate the stability of the toxicological properties of phosphorus in water. The susceptibility (LC50) of bluegill (Lepomis macrochirus) to phosphorus was evaluated under static bioassay conditions for a 96-hour period utilizing solutions which were "aged" for 0, 12, 24, 48, and 96 hours prior to initiating the toxicity tests. Fish (10 fish/replicate, 3 replicates/concentration, 30 fish/concentration) were introduced into aged test solution at each time period. The bluegill used in these tests were acquired from a commercial fish farmer in Nebraska, and had a mean (± S.D.) wet weight of 0.8 (± 0.2) g and a mean (± S.D.) standard length of 32 (+ 4) mm. The standard diluent had a pH of 7.1 and a total hardness (EDTA) of 35 mg/l as $CaCO_3$.

In order to investigate the probable degradation of phosphorus under various test conditions, one concentration of phosphorus was maintained (without fish) under each set of water quality conditions. Water samples were removed from these test vessels at 0, 24, 48 and 96 hours.

(B) FLOW THROUGH ACUTE TOXICITY TESTS - Procedures used in these toxicity tests were based on protocols described in "Methods for Acute Toxicity Tests with Fish, Macroinvertebrates, and Amphibians" (EPA, 1975) except where stated otherwise.

The flow-through bioassays were conducted using an intermittent-flow proportional dilution apparatus (Mount and Brungs, 1967). The apparatus provides for intermittent introduction of seven concentrations of the test compound into 30-liter test vessels and diluent water to a vessel serving as a control unit. The control vessel received solvent (DMSO) at a concentration equivalent to the greatest amount of DMSO introduced to any test vessel. During bioassays with fishes, the flow rate of test solutions to each 30-l aquarium was 5 l/hour throughout the test period. During the flow-through invertebrate bioassays, flow rate to each 1.75-l aquarium was 4.0 l/day. Twenty fish were randomly assigned to each test vessel within 30 minutes after the compound was added. Twenty daphnids or chironomids were randomly assigned to each replicate test chamber within 30 minutes after the phosphorus was added.

Diluent water used in these tests had the same water quality characteristics as previously described for holding water. All test vessels were maintained in water baths at 21 $\pm$ 1.0^{o}C and test solutions were not aerated during the test. During

these tests, the dissolved oxygen concentration, pH and temperature of test solutions were checked at various intervals during exposure in the highest, middle and lowest test concentrations, at a minimum. DO and temperature were measured with a YSI dissolved oxygen meter and combination oxygen-temperature probe; pH was measured with a Corning Digital pH meter and probe.

Results of the definitive tests were expressed as the time-dependent (24- and 96-hour) LC50, and time-independent (incipient) LC50, the measured concentration of test compound in diluent water which caused 50% mortality in test populations of fish and invertebrates with no additional significant response (<10%) during the final 48 hours of exposure. Test concentrations and corresponding observed percentage mortalities were converted to logarithms and probits, respectively, and these values were utilized in a least squares regression analysis. The LC50's and their 95% confidence intervals were calculated from the regression equation.

(C) CRITICAL LIFE STAGE STUDIES - The effects of 30 days continuous exposure to phosphorus on the eggs and fry of channel catfish and fathead minnows were investigated using the procedures recommended by EPA (1972). A proportional diluter (Mount and Brungs, 1967) modified with a McAllister et al. (1972)

chemical metering device delivered water and toxicant at a dilution factor of 0.5 to the test aquaria. Chemical stock solutions were dissolved in dimethyl sulfoxide (DMSO) to a volume of two liters. Stock solutions were prepared weekly. Five concentrations, a control and solvent control flowed through separate glass delivery tubes to duplicate test aquaria. Thus, each concentration and each control was replicated and each replicate was designated as either A replicate or B replicate. Test aquaria measured 30 x 35 x 30 cm. Each test aquarium contained two growth chambers (13 x 30 x 27 cm) designated A and B, which had stainless steel 40 mesh screen affixed to one end allowing water to drain out while retaining the young fish. A constant-level drain tube extending 15 cm above the bottom of each test aquarium retained a total volume of 15.75 l. Each growth chamber contained a water volume of 5.9 l. A glass, flow-splitting chamber was calibrated to deliver an equal volume to each of the growth chambers. The test water was delivered to all chambers at a mean flow rate of 13.8 tank volumes for channel catfish and 16.8 tank volumes for fathead minnows per 24 hours.

A constant temperature of 22 $\pm$ 1.0^{o}C was maintained for test organisms by placing aquaria in water baths in which circulating

water was heated by immersion coil heaters and regulated by mercury column thermoregulators.

Exposure of channel catfish eggs began within 96 hours after fertilization. Eggs were obtained from a catfish farmer in Missouri. Fifty eggs were randomly distributed to each egg cup. Eggs were treated each day during the first three days of exposure with a 3 minute dip in a 30 ppm solution of malachite green to retard fungus. Dead eggs were removed and counted each day until hatching was completed. On day 6-7 after exposure began, 25 fry were randomly selected and transferred to the larval growth chambers. Fry were fed brine shrimp nauplii ad libitum twice per day and Agway Strike Trout Starter once per day, beginning 5 days after hatching was completed and continuing throughout the larval exposure period.

Exposure of fathead minnow eggs began within 24 hours after fertilization. Eggs were obtained from brood stocks at the Aquatic Toxicology Laboratory of E G & G, Bionomics in Wareham, Massachusetts.

Their availability dictated that thirty-five eggs be randomly distributed to each egg cup. Dead eggs were removed and counted each day until hatching was completed (3-5 days after exposure began at 22°C). After hatching was completed and based on survival, 20 fry were randomly selected and transferred to the larval growth chambers. Fry were fed

brine shrimp nauplii ad libitum twice per day, beginning one day after hatching was completed and continuing throughout the exposure period.

For both species the two egg cups were oscillated in their respective test vessels by means of an egg cup rocker arm apparatus (Mount, 1968). Percentage hatch was based on the number of live fry in the egg cup when hatching was completed. After fry were transferred, growth chambers were siphoned twice weekly to remove fecal material and excess food. For each group of fry, total length was determined at 30 days post-hatch using the photographic method of McKim and Benoit (1971). Percentage survival at 30 days post-hatch, based on cumulative mortality, was also recorded at this time.

Based on acute toxicity information nominal concentrations were established for both tests. Catfish were exposed to nominal phosphorus concentrations of 10, 5, 2.5, 1.25, and 0.62 ug/l. Fathead minnows were exposed to nominal phosphorus concentrations of 5, 2.5, 1.25, 0.62, and 0.31 ug/l. In both tests, the high concentration of the solvent (DMSO) in the exposure system was calculated at 503 mg/l. In both cases, the solvent control concentration of DMSO was established at 510 mg/l.

Water was sampled from the highest, middle, and lowest phosphorus concentrations a minimum of once per week. All water samples taken for analysis of phosphorus concentration, total hardness, and pH were from the A replicates of test aquaria. Grab samples were taken at a point approximately midway between the water surface and the bottom and the sides of each larval chamber. Equivalent samples from the two larval chambers of each concentration measured were composited into one sample. Analytical samples were extracted immediately after sampling.

The means of the measured percentage hatch, and means of percentage suvival at 30 days post hatch from duplicate aquaria were transformed to the arcsin square root. Each of the means of the measured biological parameters were then subjected to analysis of variance according to Steel and Torrie (1960). When treatment effects were indicated, the means of these effects were subjected to Dunnets procedure of comparing treatment means with control (Steel and Torrie, 1960). All differences were considered statistically significant at a probability of P=0.05.

(D) INVERTEBRATE CHRONIC TESTS - To investigate the chronic toxicity of phosphorus to both midge and daphnids a proportional

diluter (Mount and Brungs, 1967) with a dilution factor of 0.5 and a syringe injector, delivered the test water and test compound to the mixing chamber, and mixing cells. From the mixing cells, the phosphorus solutions were delivered to each of 4 replicate aquaria through individual glass delivery tubes. Phosphorus stocks were prepared in DMSO. Two of the 4 control replicate aquaria received doses of DMSO identical to the greatest amount of DMSO delivered to the phosphorus treated vessels, to detect any possible DMSO related effects. The concentration of DMSO in these aquaria was 50-70 mg/l.

Each experimental unit consisted of cylindrical glass battery jars 18 cm high and 13.5 cm wide. A 3 x 8 cm notch was cut into the top edge of the aquaria and covered with Nytex R 40 mesh screen to provide drainage. Cylindrical cages constructed of aluminum 16 mesh screen were affixed to the battery jars to allow for adequate emergence areas for the C. tentans chronic exposure.

The water depth within the aquaria was 15 cm and the volume was 1.75 l. Fifty ml of solution were delivered to the individual test vessels every 25 minutes during the D. magna exposure. Fifty ml of solution were delivered to the

individual test aquaria every 8 minutes during the C. tentans exposure. For both species, the initial 3 days of the exposures were maintained under static conditions to prevent damage of the young or early instar organisms due to turbulence.

D. magna (<24 hours old), procured from laboratory stock cultures, were used to initiate the chronic exposure. Twenty D. magna were randomly assigned to each test aquarium. Observations of survival and production of young were made weekly for each test aquarium. If young were present, they were counted and discarded. On day 21, twenty young from each aquarium were retained for the initiation of the second generation exposure.

C. tentans (<48 hours old), collected as eggs from wild stock were used to initiate this chronic exposure. At least fifty organisms were placed in each test vessel. Prior to the introduction of the test organisms, the aquaria were supplied with a substrate consisting of homogenized paper towel, in water, approximately 1 cm deep, and one hundred 2 and 3 mm diameter sections of glass tubing, 2 to 3 cm long. Early instar C. tentans

utilize the paper towel substrate for construction of dwelling tubes, while late instar larvae, generally, utilize the glass tubing, where their numbers can be visibly quantitated.

Determination of the survival of larvae was made after the control animals had entered their 4th instar stage. Beginning with the onset of emergence, daily records were kept of emergence, adult survival, pupae survival, and egg production. The data were compiled up to the day at which adult mortality of the control animals was greater than emergence of these controls. At this point the aquaria were cleaned, new substrate was supplied, and the second generation was initiated with larvae introduced into the same treatment level from which they were spawned.

The food supplied in these bioassays consisted of homogenized Agway Strike trout starter food and cerophyll (20:1 ratio). The combination was blended in water and was filtered through a stainless steel 102 mesh screen for removal of large particles prior to use. A 0.2 ml aliquot of the supernatant of the food solution (35 mg/ml) was pipetted into each aquarium of the D. magna test three times daily. Aliquots ranging from 0.2 to 0.4 ml of this solution, depending upon the clarity of the water, were pipetted into each aquarium during the C. tentans chronic exposure three times daily.

Data were subjected to analysis of variance according to Steel and Torrie (1960). The data for percentage survival and percentage hatch were transformed to arcsin $\sqrt{\text{percentage}}$ prior to analysis. If significant (P=0.05) differences from the controls were observed, the data were used in the Dunnett's Procedure (Steel and Torrie, 1960) to determine which treatments differed from the controls.

(E) MIDGE/SEDIMENT/PHOSPHORUS CHRONIC STUDY - To investigate the chronic toxicity of hydrosoil containing elemental phosphorus to midges, a proportional diluter (Mount and Brungs, 1967) was employed as a means of water delivery to the test system allowing a delivery of 50 ml of diluent water (DO >7.0 mg/l, temperature 20 $\pm$ 1.0^{o}C) to each test vessel every 8 minutes. No toxicant was dispensed into this delivery system.

Aquaria consisted of cylindrical glass battery jars, 18 cm high and 13.5 cm in diameter. A 3 x 8 cm notch was cut into the upper edge of the aquaria, and covered with Nytex 40 mesh screen to provide drainage. Cylindrical cages constructed of aluminum 16 mesh screen, approximately 10 cm high and 13.5 cm wide, were affixed to the battery jars. This allowed for an emergence area, which enabled adult chrionomids to escape from the aqueous environment, yet remained confined to the specific aquarium from which they emerged.

Test concentrations of phosphorus in hydrosoil, were obtained by introducing the desired amount of P_4, in dimethyl sulfoxide into 300 g of sediment having a composition of sand (62.8%), silt (30.0%), clay (7.2%) and total organic matter (2.3%) with a pH of 6.0 and a cationic exchange capacity of 5.5 meq/100g.

The water depth within the aquaria was 15 cm and the volume was 1.75 l. The amount of DMSO introduced to the treatment vessels was also introduced to two of the control vessels in order to detect any possible effects which may be related to the presence of DMSO. Duplicate sediment samples were taken from each treatment and control on day 0, 7, 14, 21, 28, 35, 42, and 56 to quantitate P_4 in sediment.

Chironomus tentans (<48 hours old), collected as eggs from wild stock, were used to initiate this chronic exposure. At least fifty midge larvae were placed in each test vessel. After introduction of these animals, the test vessels were maintained under static water conditions for 48 hours to allow sufficient time for the larvae to inhabit the sediments. After 10-15 days exposure, upon the commencement of emergence, daily records were maintained of emergence, adult survival, and egg production. Eggs, when present, were collected, counted, fertility determined

microscopically and egg masses then were incubated in 100 ml of appropriate test solution. These data were compiled up to the day at which adult mortality of the control animals exceeded emergence of the controls. At this point, the aquaria were cleaned of fungus, dead organisms, exuvia, and remaining larvae, and the second generation exposure was initiated in the same manner as the first. This generation was initiated with the first instar larvae originating from the treatment level into which they were placed for the remainder of the test.

The food supplied in this bioassay consisted of homogenized Agway Strike trout starter food and cerophyll at a 20:1 (weight basis) ratio, respectively. This mixture was then filtered through a stainless steel 102 mesh screen for removal of large particles prior to use. Aliquots ranging from 0.2 to 0.4 ml of this solution (35 mg food/ml water) depending upon the organic enrichment of the water, were pipetted into each aquarium 5 times daily.

Data from this chronic exposure were subjected to analysis of variance according to Steel and Torrie (1960). The data for percentage survival and percentage hatch were transformed to arcsin $\sqrt{\text{percent}}$ age prior to analysis. If significant (P=0.05) differences from the controls were observed, the data were used in the Dunnett's Procedure (Steel and Torrie, 1960), to determine which treatments differed significantly from the controls.

(F) FATHEAD MINNOW CHRONIC STUDY - To investigate the chronic toxicity of phosphorus to fathead minnows, we closely followed the recommended bioassay procedures for fathead minnow chronic tests issued by the Environmental Research Laboratory, Duluth, Minnesota (U.S. EPA, 1971b).

A proportional diluter (Mount and Brungs, 1967) with a dilution factor of 0.5 was used to deliver five concentrations of elemental phosphorus and two controls to duplicate test aquaria. One control received only the diluent water (negative control) and a second control received a volume of DMSO (positive or solvent control) equal to that which was added to the aquaria containing the highest concentration of phosphorus to which fish were exposed. A stock solution of phosphorus, dissolved in DMSO was delivered to the mixing container from a 50 ml glass syringe with a stainless steel needle. The amount of DMSO added to the highest concentration of phosphorus and to the solvent control was 7.2 mg/l. This is less than 0.001 of the LC50 of DMSO to fishes as reported by Wilford (1967). A flow splitting chamber (Benoit and Puglisi, 1973) was used to promote mixing of the phosphorus and diluent water prior to delivering test water to the aquaria through glass tubing. Each glass aquarium (90 x 30 x 30 cm) was subdivided by a stainless steel 40 mesh screen to provide space for two fry chambers (30 x 12 x 35 cm) and a spawning chamber. Each aquarium was duplicated, resulting in two aquaria, four fry

chambers and two spawning chambers for each test concentration. The water level in each aquarium was maintained at 15 cm by a standpipe. The flow rate to the duplicate spawning and quadruplicate fry chambers was seven times their volume per 24 hours.

Five spawning tiles made from halved, 7.5 cm-wide sections of 10 cm diameter cement-asbestos drain tile were placed in each spawning chamber with the concave surface downward. Egg groups were incubated in "egg cups" made from 5 cm diameter glass jars with Nytex R 40 mesh screen bottoms. The egg cups were oscillated in the test water by means of a rocker-arm apparatus driven by a 2 rpm motor (Mount, 1968).

A constant temperature of 25 $\pm$ 1.0^{o}C was maintained in the aquaria by placing them in water baths within which circulating water was heated by immersion coils and regulated by a mercury column thermoregulator.

The photoperiod followed the normal daylight hours of Evansville, Indiana (U.S. EPA, 1971b) and was adjusted on the first and fifteenth day of each month beginning with the Evansville daylength of December 1st on the first day of the test as suggested in the protocol. Illumination was provided by a

combination of Durotest (Optima F.S.) and wide spectrum Grow Lux fluorescent lights located centrally, 64 cm above the surface of the water in the aquaria. The entire experimental unit was screened with black, polyethylene curtains to prevent disturbance of the fish and to minimize the effect of ambient laboratory lighting on the intended photoperiod.

Temperature and dissolved oxygen concentrations were measured daily, using a YSI dissolved oxygen meter with a combination electrode polarographic probe, in test aquaria on a rotating basis so that all aquaria were checked at least once each week. Total hardness, alkalinity, pH and acidity were measured in each concentration during the test, according to methods in APHA et al., 1971. Water samples were removed weekly from each aquarium during the first four weeks and every other week for the remainder of the test to monitor phosphorus concentrations.

Chronic exposure of fathead minnows to phosphorus began on September 9, 1975 with eggs from brood stock at the U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Two groups of fifty eggs each were incubated in each test aquarium. Dead eggs were counted and removed daily until hatching was completed (3-5 days). Percentage hatch (number of live fry/35 eggs) was calculated for each duplicate tank.

Twenty fry (<24 hours) were placed in each fry chamber and were fed brine shrimp (Artemia salina) nauplii ad libitum three times daily for the first 30 days. During the next 30 days, fry were fed frozen brine shrimp three times daily. At 30 and 60 days, percentage survival and mean total lengths were determined using a photographic method (McKim and Benoit, 1971). At 60 days post-hatch, fish from the two fry chambers in each duplicate aquarium were combined and 15 fish were impartially selected and placed in the respective spawning chamber. The remaining fish were wet weighed in groups and frozen for possible tissue analysis of phosphorus. While in the spawning chambers, fish were fed frozen brine shrimp twice daily supplemented by Daphnia magna and Agway Strike trout food granules until the study was completed. All tanks were siphoned weekly to remove fecal material and other particulates. Tanks were brushed every other month when algal growth was excessive.

By test day 150, most fish had well developed secondary sexual characteristics and the sex ratio in each aquarium was reduced by selecting three males and six females for continued exposure and discarding the remaining fish. Research at this laboratory has shown that a reduction of the total number of fish and of the ratio of males to females in the spawning chamber can result in an increased number of spawns and number of viable eggs.

When spawning began (day 155), eggs were removed from the underside of spawning tiles after 1:00 P.M. each day and counted. Fifty eggs from the first ten spawns in each aquarium were placed in individual egg cups suspended in the corresponding test water. At least three groups of control eggs were transferred to phosphorus - treated aquaria in which little or no spawning had occurred. Eggs spawned by fish exposed to phosphorus were also transferred to control tanks to assess the effects of previous exposure of parents to phosphorus on egg incubation and hatching in phosphorus-free water. Percentage hatch was calculated for each group of eggs incubated and the mean number of spawns per female, eggs per spawn and eggs per female were determined for each duplicate spawning chamber.

Twenty fry from the first two incubated spawns in each aquarium were placed in the corresponding fry chambers and fed brine shrimp nauplii ad libitum three times daily for 30 days. Fry from transferred control eggs were used in duplicates where no spawning or poor hatching had occurred. After 30 days exposure, percentage survival and total length of fry were determined photographically, and each fry group was weighed to determine average wet weight of surviving fish.

The test was terminated on day 241 after spawning had virtually ceased in all aquaria for a period of one week. Total length,

wet weight, sex and gonad condition were determined for each fish after which the fish were killed and frozen for possible tissue analysis.

Means of the measured biological parameters from duplicate aquaria were subjected to analysis of variance (completely randomized block design, P=0.05). Data for percentage survival and percentage hatch were transformed to arcsin $\sqrt{\text{percentage}}$ prior to analysis. When treatment effects were indicated these means were subjected to Dunnett's Procedure or, where there were insufficient error degrees of freedom (<5) to obtain a d' using Dunnett's test, the test for least significant difference (Steel and Torrie, 1960).

(G) BIOACCUMULATION AND ELIMINATION - The exposure system consisted of a proportional diluter modified to deliver two concentrations of phosphorus and a control to duplicate test aquaria. A mechanical injector mechanism pumped microliter quantities of the phosphorus stock solution from the syringe to the mixing container during each diluter cycle. The average number of cycles during the test was approximately 270 per day. During each diluter cycle, a one liter volume was delivered to each 68 liter aquarium providing a flow rate equal to four volume replacements per day.

Each glass aquarium measured 90 x 30 x 30 cm with a water depth of 25 cm. Aquaria rested in water baths which maintained a temperature of 24 $\pm$ 1° C. A photoperiod of 16 hours light and 8 hours of darkness was controlled by an automatic timer.

Illumination was provided by a combination of Durotest (Optima FS) and wide spectrum Grow Lux fluorescent lights.

Temperature and dissolved oxygen concentrations were measured with a YSI dissolved oxygen meter equipped with a combination electrode-polarographic probe. All aquaria were checked once each week on a rotating basis. Total hardness and pH were measured in each phosphorus concentration and control.

Measured concentrations of phosphorus in water were determined by analysis of samples taken generally once per week during the exposure period. Aquaria receiving nominal concentrations of 2.0 and 0.20 ug/l phosphorus were sampled in duplicate and a single water sample was removed from each control aquarium. Samples were extracted immediately with benzene and prepared for analysis according to methods described below. Methods for analysis of fish tissues are also described.

The test began May 25, 1977 by randomly assigning 120 channel catfish and 200 fathead minnows to separate aquaria with nominal phosphorus concentrations of 2.0 and 0.20 ug/l. Sixty channel catfish and 100 fathead minnows were assigned to separate control aquaria. All fish were fed a diet of frozen brine shrimp (Artemia salina) and Agway Strike pelleted trout food twice daily. Aquaria were cleaned of particulate matter twice per week.

Sampling of channel catfish and fathead minnows for phosphorus residues began on days 1 and 2 of exposure and continued at one week intervals through day 47. In addition to regularly scheduled samples of live fish, mortalities which were not decomposing were pooled into one sample on the day they occurred. After 47 days exposure, all channel catfish and a portion of the fathead minnows were transferred to aquaria receiving diluent water for a one week depuration period. These fish were sampled for phosphorus residues on days 2 and 7 after transfer. Fathead minnows not transferred for depuration were continued in exposure through day 77. All females which appeared sexually mature at this time were pooled into one sample from the control and phosphorus treated aquaria.

For each fish species, on each scheduled sample day, two groups of three fish were pooled from phosphorus treated aquaria and one group of three fish was removed from controls. Each sample of channel catfish was divided into muscle, liver and remaining tissues. Muscle samples were obtained by eviscerating fish and removing head, tail and fins. Fathead minnow samples were divided only into muscle and remaining tissues (including liver) due to the small size of the fish. In order to obtain sufficient biomass, ovaries were separated from mature female fathead minnows only on day 77, and sampled in addition to muscle and remaining tissue.

(H) CHEMICAL ANALYTICAL METHODOLOGY - Addison and Ackman (1970) extracted elemental phosphorus from water with benzene or isooctane, using one part of solvent to two parts of water by volume. A

portion of the solvent was analyzed by gas chromatography using flame photometric detection.

E G & G, Bionomics modified the above methods by extracting well water from test aquaria with two 1.0 ml portions of benzene: Volumes of samples were 200 ml from aquaria where the nominal concentration was $\leq$ 0.6 ppb and the control aquaria, and 100 ml of sample from concentrations >0.6 ppb. The two benzene extracts were combined and an aliquot was analyzed by gas chromatography using flame thermionic detection. The detector operates in a mode specific for either nitrogen and phosphorus or phosphorus only. The conditions of nitrogen/phosphorus detection are a relatively cool, hydrogen starved flame with rubidium vapor supplied by an electrically heated element located above the flame. In the phosphorus mode a rich hydrogen flame at high temperature completely oxidizes nitrogen and phosphorus column effluent, however nitrogen is not detected. We operated the thermionic detector in the nitrogen/phosphorus mode since this mode was ca five times more sensitive to elemental phosphorus than the high temperature phosphorus mode. No nitrogen or other interferences in the analysis were observed. Instrumental conditions were as follows:

Instrument

Perkin Elmer Model 3920 gas chromatograph with rubidium thermionic detection specific for nitrogen/phosphorus detection.

Column

6' x 2 mm ID glass packed with 5% Dexsil 300 GC coated on 60/100 mesh Supelcoport

Temperatures

Column - 170°C

Inlet - 230°C

Outlet - 240°C

Gas Flows

Column - 30 cc N_2/min

Reactant - 7 cc H_2/min and 100 cc air/min.

Reorder

Leeds & Northrup, 0-1 mV full scale response

0.5 cm/min chart speed

Response

0.5 ng of elemental phosphorus produced half-scale response at 1X1 and eluted in 2.1 minutes

Solutions of known elemental phosphorus concentrations were prepared by weighing elemental phosphorus (Fisher Cat. No. P-104, lot no. 767980) under water and rapidly transferring the phosphorus to a known volume of benzene solvent. Dilutions of the stock solution were used to calibrate the gas chromatograph prior to sample analyses. The stability of a low concentration of elemental phosphorus and hence the integrity of the analytical scheme was verified by analyzing 0.26 ppm phosphorus solution, which was prepared on June 24, 1975; and a freshly prepared 0.21 ppm phosphorus solution, which was prepared on July 25, 1975. Both

solutions were analyzed on July 25, 1975. The analytical results indicated that the standard which was stored at ambient room conditions for 30 days contained 7 percent less elemental phosphorus than the freshly prepared standard. Therefore, all water samples were extracted immediately upon collection and the extracts were analyzed within two weeks with no correction factors applied to the calculation of results.

The recovery of elemental phosphorus from water by the above method was virtually complete; therefore, no correction factor was utilized in calculations to account for method loss. The complete extraction of elemental phosphorus was verified by separately analyzing the first, second, and third benzene extraction of test aquaria samples (Table 1).

Having demonstrated that low concentrations of elemental phosphorus in benzene are relatively stable and that two benzene extractions of water effectively remove all elemental phosphorus present in the water, the assumption that the overall recovery of the method was 100%, was valid.

Samples of fish tissues were extracted by homogenizing with a 25 ml volume of benzene for tissue masses ca. 2-7 grams, or a 15 ml volume of benzene for tissue masses of ca 2 grams or less, using a PT-10 ST Willems Polytron. The duration of the homogenization was considered a critical variable and was maintained at 30-45 seconds.

To avoid unnecessary transfer, the extraction was carried out using a 40 ml centrifuge tube. The sample was centrifuged for 5-10 minutes to settle the solids, and a 10 ml volume of the benzene extract removed for analysis by gas chromatography, using the following instrument conditions:

Instrument

Hewlett-Packard 5840A gas chromatograph equipped with a flame ionization detector operating in the phosphorus mode and a model 7671A automatic sampler.

Column

6' x 2 mm ID glass column packed with 3% Dexsil on 80/100 mesh Supelcoport.

Temperatures

(C^{O}): Column - 77

Inlet - 200

Detector - 210

Gas Flow

50 cc/min., Nitrogen Carrier

50 cc/min., Air

4.5 cc/min., Hydrogen

Set Points

Slope sensitivity- 0.02

Area rejection - 50

Cycle time - 7.0 minutes

Calibration method - external standard

% retention window - 5.0

Retention Time

5.0 minutes

Daily instrument calibration was performed using 0.047 and 0.0047 mg/l phosphorus in benzene standards. Standards were checked after every eighth sample during an automatic sampling sequence.

Quality control samples were produced by adding a 1.0 ml volume of 0.16, 1.9 and 16 ug/l phosphorus in acetone standards respectively to weighed channel catfish and weighed fathead minnows. Fish of each species were also left unfortified and analyzed as blanks.

Quality control fish samples were extracted and analyzed according to the above procedure with the following analytical results:

Sample I.D.		Sample Weight, g	Phosphorus Added, ug	Phosphorus Recovered, ug	% Recovered
CCF-Blank	A	0.9186	-	0.015	-
	B	1.1374	-	0.015	-
	C	1.2481	-	0.030	-
CCF-low	A	0.6167	0.16	0.17	106
	B	0.4992	0.16	0.17	106
	C	0.5496	0.16	0.17	106
CCF-Middle	A	0.7274	1.9	2.2	116
	B	1.1269	1.9	2.2	116
	C	1.1269	1.9	2.2	121
CCF-High	A	0.7337	16	18	113
	B	0.6334	16	18	113
	C	0.5809	16	18	113

FHM-Blank	A	0.7099	-	0.018	-
	B	0.3828	-	0.018	-
	C	1.0083	-	0.018	-
FHM-Low	A	0.7237	0.16	0.16	100
	B	0.2478	0.16	0.14	86
	C	0.1743	0.16	0.16	100
FHM-Middle	A	0.1604	1.9	2.0	105
	B	0.4776	1.9	1.9	100
	C	0.4453	1.9	1.9	100
FHM-High	A	0.4858	16	15	94
	B	0.2629	16	17	106
	C	0.6471	16	17	106

Average CCF Recovery: 112.2 $\pm$ 5.3 %

Average FHM Recovery: 99.7 $\pm$ 6.4 %

The minimum detectable concentration of phosphorus in fish tissue was 0.0075 ug with a 15 ml extraction volume. A percentage recovery of 100 per cent was assumed for all samples.

RESULTS

(A) STATIC ACUTE TOXICITY TESTS - The bluegill was the most sensitive of the aquatic organisms exposed to phosphorus (Table 2). The fishes were an order of magnitude more sensitive to phosphorus than were most of the invertebrates and the phytoplankton during static bioassay (Tables 2,3,4). The exception to this generalization was observed with daphnids, the sensitivity of which was very similar to the fishes. The 96-hour LC50 values for the four fishes tested and the 48-hour EC50 value for daphnids based on nominal concentrations of phosphorus ranged from 6-73 ug/l. Comparable EC50 values for the other invertebrates and concentrations obviously effecting the phytoplankton species ranged from 70->670 ug/l.

Exposure to phosphorus had a varied effect on the phytoplankton tested, inhibiting the growth of S. capricornutum and N. pelliculosa but having a stimulating effect on the growth of M. aeruginosa and A. flos-aquae. This pattern of effects of exposure to phosphorus was indicated by both number of cells (as determined by count) or optical density (Table 3) and chlorophyll a content (Table 4). There was, however generally no clear dose-response relationship evident over the range of concentrations tested, an observation which is not unusual when working at concentrations at or near maximum solubility where

nominal differences in concentration may or may not be real. In view of the lack of any obvious dose-response relationship for phytoplankton, no attempt to calculate EC50 values is feasible.

The acute toxicity of phosphorus to various life stages of the fathead minnow suggested an increasing sensitivity to phosphorus with age (Table 5). The eggs of this species were relatively insensitive to phosphorus exposure while the sensitivity of 1-hour-old fry was <7-day fry, which was <30-day fry, which was <60-day fry.

The results of the bioassays to determine the effects of varying water quality on the toxicity of elemental phosphorus to bluegill indicate that temperature had no effect on the acute toxicity of phosphorus (Table 6). The 96-hour LC50 values for phosphorus and bluegill over a temperature range of 15-25°C varied from 2-5 µg/l. Similarly, alkaline pH and moderate water hardness (100 mg/l as $CaCO_3$) had no effect on acute toxicity of phosphorus. However, acid pH (6.0) and very hard water (250 mg/l as $CaCO_3$) decreased the acute toxicity of elemental phosphorus to bluegill by approximately an order of magnitude.

Aging solutions of elemental phosphorus in static systems for periods up to 96 hours had no effect on the toxicity to bluegill (Table 7).

The effects of varying water quality and the effects of aging on the toxicity of phosphorus solution to bluegill are not readily understood on the basis of the stability of elemental phosphorus in aqueous solution. The concentrations of phosphorus in bioassay test vessels (without bluegill) under various water quality conditions generally decreased by approximately 50% after 48 hours (Table 8). As was indicated above, however, this did not result in any observable differences in acute toxicity (Table 7). Furthermore the most rapid rate of decrease in phosphorus concentrations was observed at 25°C where the phosphorus concentration decreased by more than 85% within the first 48 hours and by more than 97% after 96 hours. Despite the rapid decrease in phosphorus concentrations, these solutions were slightly more toxic to bluegill than solutions at lower temperature where the rate of decrease was slower (Table 6).

(B) MEASURED CONCENTRATIONS OF PHOSPHORUS DURING DYNAMIC TOXICITY TESTS - The results of gas chromatographic analysis of water samples to quantitate phosphorus concentrations during dynamic bioassays, both acute and chronic, indicated that measured concentrations were only occasionally equivalent to nominal. Mean measured concentrations were generally 50-80% of nominal concentrations.

(C) FLOW-THROUGH ACUTE TOXICITY TESTS - Dissolved oxygen values during dynamic bioassays were >60%, >75%, and >90% for fishes, daphnids, and midges, respectively. Elemental phosphorus was only slightly more toxic to midges under continuous dynamic exposure (at 48 hours) as compared to static exposure. For example, after 48 hours the EC50 for midges was 140 ug/l under static conditions (Table 2) and 111 ug/l under dynamic conditions (Table 9). Phosphorus appeared even less toxic to daphnids at 48 hours of exposure under dynamic conditions as compared to static conditions; however, P_4 concentrations in both cases are nominal. On the other hand, the toxicity of elemental phosphorus to fishes during dynamic continuous exposure was much greater than during static exposure. Incipient LC50 values for both fishes tested were at least an order of magnitude less than 96-hour LC50 values determined during static bioassays. Furthermore the duration required to estimate the incipient LC50 for phosphorus to catfish (624 hours) clearly indicates the cumulative nature of phosphorus toxcitity to fishes during continuous exposure. After five unsuccessful attempts to conduct a fathead minnow dynamic study, this effort was terminated.

(D) TOXICITY TO CRITICAL LIFE STAGES - Continuous exposure to elemental phosphorus during the period of egg incubation had no significant effect on the percentage hatch of channel catfish eggs (Table 10). Beginning on the fifth day of exposure we observed that the embryos and newly hatched fry exposed to 6.8 and 5.0 ug/l phosphorus appeared abnormal in coloration.

These fish appeared jaundiced in color, lacking the red coloration that was characteristic of the circulatory systems in fish exposed to lower concentrations of phosphorus and the controls. Percentage survival of catfish fry at 30 days post hatch was significantly reduced by exposure to 6.8 ug/l phosphorus. Mortality of these fry was observed throughout the exposure period with the greatest incidence of mortality during the last four days of exposure. Furthermore, exposure to 6.8 ug/l significantly reduced total length of catfish fry at 30 days post hatch. No significant effects of exposure of catfish fry to 5.0 ug/l were observed.

The percentage of fathead minnow eggs hatching was similar among controls and eggs exposed to phosphorus concentrations as high as 6.8 ug/l (Table 11). Also, the percentage survival of fry at 30 days post hatch was unaffected by continuous exposure to phosphorus concentrations as high as 6.8 ug/l. However, mean total length of fry was significantly reduced when compared to controls by continuous exposure to 6.8, 2.5, and 1.5 ug/l phosphorus. No significant effects of exposure to 0.7 ug/l phosphorus were observed for any of the parameters measured during the exposure of fathead minnow eggs and fry to elemental phosphorus.

(E) CHRONIC TOXICITY TO INVERTEBRATES - No empirical or statistical differences between controls and solvent controls for any of the parameters measured were observed with either daphnids

or midges. Therefore, all data for controls represent pooled data for the two replicates of both solvent and non-solvent controls. Continuous exposure of daphnids to 8.7 ug/l elemental phosphorus significantly reduced survival at all intervals during the first generation exposure (Table 12). The number of young produced per parthenogenetic female was also significantly reduced during the first generation of exposure to 8.7 ug/l phosphorus (Table 13). No significant effects on survival of first generation daphnids or number of young produced per parthenogenetic female were observed during exposure to phosphorus at concentrations $\leq$6.9 ug/l. Due to the low survival and low reproduction of daphnids exposed to 8.7 ug/l, no second generation exposure at this treatment was possible. During the exposure of second generation daphnids, no significant effects of exposure to concentrations of phosphorus $\leq$6.9 ug/l were observed. Based on the reduced survival and production of young per parthenogenetic female *D. magna* during this chronic exposure, the estimated minimum threshold concentration of phosphorus to this species is >6.9<8.7 ug/l.

After six unsuccessful attempts to complete a full two generation chronic toxicity study with the midge (*Chironomus tentans*), this effort was terminated. Generally all efforts were hampered by poor survival and poor production of fertile egg masses. Despite the problems which precluded the successful completion of full chronic exposure, we were able to make some significant valid

scientific observations concerning the toxicity of elemental phorphorus to midges. For example, continuous exposure of midge egg masses and developing larvae for 8 days to concentrations of phosphorus ranging from 0.14-2.0 ug/l significantly reduced survival of midges when compared to controls (Table 14). Since all concentrations significantly effected survival of midges within 8 days, a second exposure of midges to a series of phosphorus concentrations ranging from 0.005-0.067 ug/l was conducted for one generation (28 days). Continuous exposure to concentrations of elemental phosphorus $\leq$0.067 ug/l had no significant effect on survival of larvae and pupae, on survival and emergence of adult midges, or on the number of eggs produced per adult (Table 15). Unfortunately none of the egg masses produced among any of the treatments or controls were fertile suggesting that copulation of adults was not occurring.

(F) TOXICITY OF PHOSPHORUS LADEN SEDIMENT TO MIDGES - Due to an error in preparation of sediment samples for phosphorus analysis, no data on mean concentrations in sediment are available. Therefore, a discussion of the experimental results are based on nominal concentrations at initiation of the experiment (Day 0). The presence of elemental phosphorus in hydrosoil at nominal concentrations of 2-500 ug/kg appeared to cause a delay in the rate of development of midges. At a period approximately midway through the normal emergence period for midges (Day 19) the percent

of adult midges emerged in the control was significantly higher than all phosphorus treatments (Table 16). However, by the end of the first generation exposure (Day 23), the percent emergence and survival was similar to controls in all phosphorus treatments. The percent emergence and survival of all experimental groups was extremely poor. The midge population inhabiting the hydrosoil spiked with 500 ug/kg phosphorus did not produce any eggs. The number of eggs produced per adult among all other treatments did not differ significantly from the controls.

The second generation exposure to the sediment spiked with 500 ug/kg was initiated with eggs transferred from the first generation controls. Analysis of the percent emergence and survival of midges during the second generation indicated, as observed during the first generation, that percent emergence and survival was similar to controls among all treatment groups. Also, as observed during the first generation, second generation midges inhabiting sediments spiked with 500 ug/kg phosphorus produced no eggs.

(G) CHRONIC TOXICITY TO FATHEAD MINNOWS - The results of the analyses for water quality parameters measured throughout the chronic exposure indicated that hardness, alkalinity, acidity, dissolved oxygen and pH varied minimally among exposure aquaria and with time during the test. The mean and range of each parameter are reported in Table 18.

The results of gas chromatographic analyses for phosphorus of water samples which were taken during the chronic exposure indicated that the mean measured phosphorus concentrations were generally 60 to 80% of the nominal concentrations selected and that the dilution factor was reasonably close to the intended 0.5 (Table 19).

Percentage hatchability of fathead minnow eggs and percentage survival of first generation fry after 30 days were not affected by exposure to phosphorus concentrations as high as 6.0 ug/l (Table 20). Total lengths of fry after 30 and 60 days exposure to 6.0, 3.4 and 1.5 ug/l phosphorus were significantly reduced when compared with control fish. At day 60, those fry exposed to 6.0 ug/l phosphorus were approximately one-half the size of the control fry (Figure 1). After 60 days, survival of fry exposed to 6.0 ug/l was significantly lower than survival of control fry and those exposed to lower phosphorus concentrations. Between days 60 and 150 of exposure, after fish were thinned to 15 per tank, all fish exposed to 6.0 ug/l had died and a significant reduction in survival had occurred among fish exposed to 3.4 and 1.5 ug/l of phosphorus. On day 150, when final spawning ratios were established for each tank, all fish which survived exposure to 3.4 and 1.5 ug/l phosphorus were severely stunted in growth and sex could not be determined by external features.

At the time adult fish were terminated (Day 241), all remaining fish exposed to 3.4 ug/l had died and a further reduction in

survival had occurred among fish exposed to 1.5 µg/l of phosphorus (Table 21). When examined, all surviving fish exposed to 1.5 µg/l phosphorus remained severely stunted in growth and no physical evidence of sexual maturity could be found externally or internally. Total length and wet weight of male fish at concentrations $\leq$ 0.71 µg/l of phosphorus were not significantly different from that of controls. Total length and wet weight of female fathead minnows exposed to 0.71 and 0.40 µg/l phosphorus were significantly reduced when compared with female fish in controls and solvent controls. The number of spawns, total eggs, spawns per female, eggs per female and eggs per spawn did not differ significantly between control fish and fish exposed to 0.71 and 0.40 µg/l. Spawning was lower in the A duplicates of fish exposed to 0.71 µg/l phosphorus but the response observed in the B duplicates was similar to controls.

Evidence of extremely cumulative effects due to phosphorus exposure was demonstrated by the poor hatchability of eggs spawned by fathead minnows exposed to concentrations of 0.71 and 0.40 µg/l (Table 22). Nearly all eggs spawned by fish exposed to these low concentrations failed to hatch when incubated in the respective exposure aquaria. When egg groups from these aquaria were incubated in control aquaria, percentage hatchability was extremely low. Percentage hatchability of eggs spawned by minnows in control and solvent control was excellent. As would be expected from first generation egg incubation, the percentage hatchability of control eggs transferred to phosphorus concentrations as

high as 6.0 ug/l was comparable to hatchability of eggs from control and solvent control.

After 30 days exposure to phosphorus concentrations as high as 6.0 ug/l, survival of fry from transferred control eggs was similar to survival of fry in control and solvent control. Total lengths of fry exposed 30 days to phosphorus concentrations as low as 0.40 ug/l were significantly less than those of fry in control and solvent control. Wet weights of fry were significantly less than those of fry in the control or solvent control at concentrations ranging from 0.71 - 6.0 ug/l.

Based on the reduced survival of fathead minnows exposed to 6.0, 3.4 and 1.5 ug/l phosphorus and on the reduced hatchability of eggs spawned by minnows exposed to 0.71 and 0.40 ug/l, the minimum threshold concentration of phosphorus to this species is <0.40 ug/l.

(H) BIOACCUMULATION AND ELIMINATION - Mean measured aqueous concentrations of phosphorus during the exposure period were reasonably close to the intended levels of exposure (Table 23). The degree of fluctuation in measured concentrations probably relates directly to the use of a diluter and exposure aquaria which were "open" to the atmosphere.

In the control and 0.20 ug/l phosphorus, channel catfish and fathead minnows appeared healthy during the entire exposure. Mortality of both species in these aquaria was negligible (<2 percent). In contrast, both species were adversely

effected by exposure to 2.0 ug/l phosphorus. All fathead minnows exposed to this concentration died between days 11 and 24. Approximately one third of the catfish initially exposed to 2.0 ug/l died between days 24 and 47. Both species developed an apparent edema of tissues particularly around the eyes and abdomen. Other symptoms included jaundiced livers and bright green or yellow intestines.

Initial accumulation of phosphorus was rapid in channel catfish exposed to 1.8 ug/l (Table 24). Concentrations 100 times the amount in water were accumulated in muscle and remaining tissues (other than liver) within two days. The bioaccumulation factor in these tissues remained relatively constant (50-100x) during 47 days exposure including two mortalities which were sampled on day 34. An apparent increase of phosphorus in muscle concentrations on day 14 is suspect, due to the poor replication (140, 710 ug/kg) of the duplicate samples which were analyzed. Mortalities often occurred during the night, and the low concentrations observed in dead fish on days 33 and 37 may reflect the degree of decomposition which occurred prior to sampling of fish. Concentrations of phosphorus in the liver were slightly lower (30-60x) than in other tissues.

Concentrations of phosphorus in catfish were proportional to the concentrations in water (tables 24 and 25).

These concentrations were barely detectable in the larger samples but suggested that fish exposed to 0.18 ug/l had concentrated 50 to 100 times this amount.

Maximum accumulation of phosphorus in fathead minnows exposed to 2.2 ug/l occurred after two days of exposure (Table 26). Bioconcentration in muscle and remaining tissues was 160 and 200 times the concentration in water at this time. After 7 days exposure tissue concentrations were lower than those observed on day 2. Fathead minnows began dying on day 12, but phosphorus concentrations in dead and dying fish were generally much lower than those observed previously.

Phosphorus concentrations in fathead minnows exposed to 0.15 ug/l were not large enough to be detected in pooled samples of 3 fish (Table 27). On day 77, a pooled sample of nine female fish contained phosphorus concentrations which were 127 and 200 times the mean concentration in water in the muscle and the remaining tissues, respectively. Ovaries which were removed from these sexually mature females contained less than 31 ug/kg phosphorus.

None of the fathead minnows and channel catfish which were removed from the control aquaria contained detectable amounts of phosphorus. Generally, little or no phosphorus was detected in muscle or liver tissues two days after fish were transferred to control water. The measured concentration in the remains of channel catfish exhibited a rapid depuration between days 47 of exposure and 2 of depuration and were not detectable by day 7 of depuration.

DISCUSSION AND CRITERIA FORMULATION

The range of incipient LC50 values estimated during dynamic bioassays which we report here for five freshwater organisms (0.6-20.0 ug/l) compares favorably with the range reported for marine animals (3.0-40.0 ug/l) by Zitko et al. (1970) based on the results of static bioassays. It is not surprising that these authors reported "no clear indication of an incipient lethal level for phosphorus and herring were found" during 160 hours exposure, since we report that incipient LC50 values for catfish could only be estimated after 624. Our findings on the acute toxicity of phosphorus to these two fishes appear to confirm the cumulative toxicity of phosphorus to fishes suggested by Zitko et al. (1970). The fact that the acute toxicity (48-hour LC50) of phosphorus to midges and daphnids is essentially equal under both static and flow-through test conditions is unexpected in view of the apparent cumulative toxicity of phosphorus to aquatic organisms. However, the observation is consistent with those of Zitko et al. (1970), who reported that the incipient lethal level of phosphorus to the beach flea (Gammarus oceanicus) was 3-4 mg/l in still (static) water and about 6.5 mg/l in moving (dynamic) water.

Several investigators have reported on the bioconcentration of elemental phosphorus in tissue of marine fishes and invertebrates (Dyer et al., 1970; Fletcher et al., 1971; Fletcher, 1974; and Maddock and Taylor, 1976). All of these

investigators reported relatively rapid accumulation of phosphorus in tissues during short-term exposures with concentrations of phosphorus in tissues by several orders of magnitude above the concentration in the water to which these animals were exposed.

The concentrations of phosphorus in the muscle of channel catfish and fathead minnows exposed to 1.8 and 2.2 ug/l in our laboratory studies are comparable to those reported in previous tests. Dyer et al. (1970) reported 71 ug/kg in the muscle of cod exposed to 1 ug/l.

Catfish exposed to 1.8 ug/l phosphorus concentrated only 60 times this amount in liver tissue. This was unexpected in view of the data which indicates that phosphorus can be concentrated a thousand fold or more in the liver of cod (Dyer et al. 1970, Fletcher 1974). However, cod may not be a representative species due to the high lipid content and liver oils which are characteristic of them. The livers of the Atlantic salmon contained less than 100 times the amount in water during exposures which parallelled those of cod (Fletcher, 1974). Bluegill exposed 16 hours to 46 ug/l phosphorus concentrated only 44 times this amount in liver tissue (Pearson et. al. 1976).

The only comparative data on phosphorus accumulation in the same species (Ictalurus punctatus) was reported for fish collected

from Yellow Lake, Arkansas (Pearson et. al. 1976). Channel catfish were collected both before and after a rain which was known to increase phosphorus contamination from a munitions filling operation. The livers of catfish collected after the rain contained a concentration of phosphorus (138.75 ug/kg), nearly identical to the maximum concentration (120 ug/kg) which we observed in our laboratory exposed fish.

The absence of significant phosphorus accumulation (<31 ug/kg) in the ovaries of female fathead minnows indicates that this was probably not the cause of the poor egg hatchability observed in the chronic test. Fletcher (1974) has suggested that tissue damage may be related to the amount of phosphorus metabolized or oxidized in the fish as opposed to the amount found in tissues.

We did not perform histological examinations of sexually mature fish which were exposed to phosphorus. Such examinations may have revealed damage to male reproductive tissues (i.e. sterility) which would preclude the production of viable eggs.

The cumulative mortality pattern observed with channel catfish fry during the critical life stage exposure to phosphorus and with fathead minnows during the chronic study suggests that while we observed no continuing process of bioconcentration of phosphorus in the tissue of fish, there is an apparent continuing process affecting target organs.

The generally accepted expression for that concentration of a water quality constituent reasonably judged to preclude hazard to aquatic organisms due to the presence of that constituent is a water quality criterion.

In general, water quality criteria specify concentrations of water constituents which when not exceeded will protect one organism, an organism community, or a prescribed water quality use with an adequate margin of safety during continuous exposure.

The procedure for the genesis of valid water quality criteria has been defined (National Technical Advisory Committee, 1968; National Academy of Sciences, National Academy of Engineering, 1973; U.S. Environmental Protection Agency, 1976). Water quality criteria reflect a knowledge of the capacity for environmental accumulation, persistence, and effects of specific toxicants on specific aquatic ecosystems. The generally accepted approach to the development of such criteria is to generate both information on the acute toxicity of a chemical to a broad range of representative aquatic organisms and an understanding of the relationship between the acute and chronic toxicity of the chemical to aquatic organisms (i.e., develop a specific application factor for estimating chronically safe concentrations for an organism based on available acute toxicity information).

However, the variability in the apparent relationship between acute and chronic toxicity of phosphorus to different aquatic

organisms appears to preclude this approach. For example, if one were to calculate a range on the estimated application factor specific for phosphorus based on the acute (30 ug/l) and chronic (>6.9<8.7 ug/l) toxicity of phosphorus to daphnids the estimated application factor would range from 0.23-0.29 (6.9/30-8.7/30). However, if one were to estimate the application factor based on the acute (140 ug/l) and chronic (0.067-0.140 ug/l) toxicity of phosphorus to midges, the estimated application factor would range from 0.0005-0.001. Finally, if one were to estimate the application factor based on the acute (20 ug/l) and chronic (<0.40 ug/l) toxicity of phosphorus to fathead minnows the estimated application factor would be <0.02. Obviously these data suggest that for whatever reasons a single application factor describing the relationship between the acute and chronic toxicity of phosphorus to aquatic organisms can not be utilized in a hazard evaluation process.

Interestingly, this situation is contrary to the apparent situations for other chemicals where the chronic toxicity to a variety of aquatic organisms has been studied. For example, Macek et al. (1976a) reported that an application factor of 0.01 accurately described the relationship between the acute and chronic toxicity of the herbicide atrazine to five of the six aquatic organisms studied. Macek et al. (1976b) also reported that an application factor of 0.1-0.3 accurately described the relationship between the acute and chronic toxicity of lindane to four of the six aquatic organisms studied. Macek et al. (1976c) have found

similar estimated application factors for acrolein, heptachlor and trifluralin with two species of aquatic organisms. Most recently, Bentley et al. (1977) have reported that an application factor of 0.02 accurately describes the relationship between the acute and chronic toxicity of nitroglycerine to three aquatic organisms (daphnids, midges, fathead minnows) studied during acute and chronic exposures.

One can speculate that for atrazine, lindane, nitroglycerine, acrolein, heptachlor (and to a lesser extent trifluralin) the data suggest the toxicity of this material is not very cumulative and there appears to be no evidence with these chemicals to suggest more than one mode of action is involved. On the other hand, phosphorus clearly is cumulative in its toxicity, unlike the above mentioned chemicals phosphorus has an obvious effect on successive generations and it appears more than one mode of action may be involved. It may be for this and other reasons that a single application factor may not be applicable to diverse aquatic organisms.

In any event, an alternate approach to recommending a water quality criteria for phosphorus for the protection of freshwater aquatic life is obviously required. A reasonable alternate approach is a complete evaluation of empirical data to identify the lowest concentration of phosphorus producing a significant harmful effect on the most sensitive species tested. Having identified this ambient concentration, a conservative approach would be to then apply a

reasonable safety factor to account for other potentially more sensitive species not tested.

A review of the data in the scientific literature and that presented in this report shows that the two species most sensitive to phosphorus tested to date are the fathead minnow and the midge. In view of the problems encountered in the midge studies, the degree of confidence in the midge data relative to the fathead minnow chronic data dictates that we utilize the fathead minnow data as a basis for criteria derivation. During a chronic exposure to phosphorus over one complete life cycle and the critical life stage of the succeeding generation we were unable to identify a no-effect concentration for fathead minnows. Significant mortality of second generation fathead minnow embryos was caused by exposure of parental fish to 0.40 ug/l, the lowest concentration tested. Thus, it is not possible to calculate an appropriate application factor to be used with acute toxicity data to derive a criterion. In lieu of that approach, a conservative estimate of a water quality criteria for elemental phosphorus would be to apply a safety factor (0.1) to the lowest observed effect level during the chronic exposure (0.40 ug/l), generating a concentration of 0.04 ug/l.

Based on the available data and the philosophy described above for the derivation of a water quality criteria for phosphorus, we recommend an ambient water quality criteria of 0.04 ug/l for the protection of freshwater aquatic life.

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Table 1 -- Analyses of consecutive 1 ml benzene extracts of 200 ml of well water containing elemental phosphorus (P_4).

Sample ID	A-74	A-75	A-76	A-79	A-80	A-81
Nominal Concentration (ug P_4/l)	7.5	3.2	1.3	7.5	3.2	1.3
	Analytical Results (ug P_4/l)					
First Extraction	7.7	2.3	0.84	8.5	2.9	1.3
Second Extraction	0.29	ND[a]	ND	0.81	0.25	ND
Third Extraction	ND	ND	ND	ND	ND	ND
Total concentration Found (ug P_4/l)	8.0	2.3	0.84	9.3	3.2	1.3

[a] ND - not detected. The minimum detectable concentration was 0.05 ug P_4/l.

Table 2 -- Acute toxicity values[a] (ug/l) for elemental phosphorus utilizing aquatic invertebrates and fishes determined during static bioassays. (nominal concentrations)

Species	Hours of exposure 24	48	96
Daphnia magna (water flea)	34 (28-41)[b]	30 (25-37)	-
Gammarus fasciatus (scud)	>420<560	250 (190-310)	-
Asellus militaris (sowbug)	>560	>560	-
Chironomus tentans (midge)	260 (210-330)	140 (110-190)	-
Lepomis macrochirus (bluegill)	27 (22-32)	9 (5-16)	6 (4-9)
Salmo gairdneri (rainbow trout)	61 (39-98)	28 (16-48)	22 (15-32)
Ictalurus punctatus (channel catfish)	152 (99-232)	87 (69-109)	73 (53-99)
Pimephales promelas (fathead minnow)	101 (73-141)	34 (24-46)	20 (16-25)

[a]Acute toxicity values are expressed as effective concentrations causing immobilization (EC50) after 24 and 48 hours for invertebrates and lethal concentrations (LC50) after 24, 48 and 96 hours for fishes.

[b]95% confidence interval.

Table 3 -- Percent change[a] in the cell density[b] of Selenastrum capricornutum, Microcystis aeruginosa, Anabeana flos-aquae, and Navicula pelliculosa after 96 hours exposure to elemental phosphorus.

Nominal Phosphorus Concentration (ug/l)	S. capricornutum	M. aeruginosa	A. flos-aquae	N. pelliculosa
7	NT[c]	0	11	-6
70	NT	0	+10	-49
200	-4	+20	+23	-58
270	-2	+31	+21	NT
340	-10	+72	+26	-54
420	-20	+58	+31	NT
530	-23	+41	+41	NT
670	-63	+48	+24	-63

[a]Percent change is relative to control cultures.

[b]Determined by cell counts for all species except A. flos-aquae which was determined by optical density.

[c]Not tested at this concentration.

Table 4 -- Percent change[a] in the chlorophyll a content of *Selenastrum capricornutum*, *Microcystis aeruginosa*, *Anabeana flos-aquae* and *Navicula pelliculosa* after 96 hours exposure to elemental phosphorus.

Nominal Phosphorus Concentration (ug/l)	*S. capricornutum*	*M. aeruginosa*	*A. flos-aquae*	*N. pelliculosa*
7	NT[b]	0	+32	-18
20	NT	NT	NT	-56
70	NT	+10	+60	-59
200	-7	+19	+58	-79
270	-9	+40	+68	NT
340	-10	+45	+63	-97
420	-24	+36	+49	NT
530	-39	+37	+51	NT
670	-68	+25	+62	-94

[a]Percent change is relative to control cultures.

[b]Not tested at this concentration.

Table 5 -- Acute toxicity of elemental phosphorus to selected life stages of fathead minnows (Pimephales promelas) as determined during static bioassays. (nominal conc.)

Life Stage	LC50 (ug/l) 24-hour	48-hour	96-hour
Eggs	>560	>560	>560
1-hour post hatch	154 (96-247)[a]	154 (96-247)	154 (96-247)
7-days post hatch	93 (53-165)	75 (54-104)	74 (54-103)
30-days post hatch	26 (18-38)	25 (17-37)	21 (11-28)
60-days post hatch	27 (22-32)	21 (17-25)	18 (15-22)

[a]95% confidence interval.

Table 6 -- Acute toxicity of elemental phosphorus to bluegill (Lepomis macrochirus) under varying conditions of water quality during static bioassays. (nominal conc.)

Temperature (°C)	pH	Hardness (mg/l $CaCO_3$)	96-hour LC50 (ug/l)
15	7.0	35	5 (3-6)[a]
20	7.0	35	5 (4-7)
25	7.0	35	2 (1-4)
20	7.0	35	5 (3-6)
20	7.0	100	4 (2-6)
20	7.0	250	86 (74-101)
20	6.0	35	69 (55-84)
20	7.0	35	8 (5-11)
20	8.0	35	4 (3-6)

[a] 95% confidence interval.

Table 7 -- Acute toxicity of aged solution of elemental phosphorus to bluegill (Lepomis macrochirus) during static bioassays. (nominal concentration)

Age of Solutions prior to bioassay (hrs).	96-hour LC50 (ug/l)
0	4 (2-7)[a]
12	4 (3-6)
24	6 (4-8)
48	5 (3-7)
96	9 (6-12)

[a]95% confidence interval.

Table 8 -- Concentrations of phosphorus in static bioassay test vessels (without fish) during 96-hour aging of 40 ug/l solution under various water quality conditions.

Temp/pH/Hardness (^{o}C) (mg/l $CaCO_3$)	Measured phosphorus concentration (ug/l) Hours of aging						
	0	4	8	24	48	72	96
15/7.0/35	40	42	28	31	25	23	16
20/7.0/35	38	38	31	18	15	13	8
25/7.0/35	41	34	28	16	6	2	1
20/6.0/35	41	38	32	28	17	14	7
20/7.0/35	38	38	31	18	15	13	8
20/8.0/35	37	38	33	23	15	13	8
20/7.0/35	38	38	31	18	15	13	8
20/7.0/100	37	39	24	22	15	13	7
20/7.0/250	36	42	35	24	15	14	8

Table 9 -- Acute toxicity of elemental phosphorus to fishes and aquatic invertebrates during dynamic bioassays.

Species	LC50[a] (ug/l) 24-hour	48-hour	96-hour	Incipient[b]
Daphnia magna (water flea)	>50	>50	-	11 (5-24)[c]
Chironomus tentans (midge)	>240	111 (31-399)	-	20 (4-99)
Lepomis macrochirus (bluegill)	>3.2		2.4 (1.7-3.5)	0.6 (0.4-1.1)
Ictalurus punctatus (channel catfish)	>19		>19	4.2 (3.3-5.4)

[a] LC50 values are based on nominal concentration for water flea, midge and bluegill and on mean measured concentrations for channel catfish.

[b] Incipient LC50 estimated after 192 hours for water flea, 120 hours for midge, 192 hours for bluegill, 624 hours for catfish.

[c] 95% confidence interval.

Table 10 -- Mean percentage hatch of channel catfish (Ictalurus punctatus) eggs, and mean percent survival and mean total length of fry at 30 days post hatch during continuous exposure to replicate concentrations of elemental phosphorus in water.

Measured Phosphorus Concentration (ug/l)	Replicate	Hatch (%)	30 Days Post Hatch	
			Survival (%)	Total Length (mm)
6.8	A	86	18 [a]	17 ± 1 [b]
	B	72	20	18 ± 2
5.0	A	90	84	20 ± 2
	B	86	60	19 ± 2
3.6	A	83	100	20 ± 2
	B	80	98	21 ± 2
1.7	A	100	92	21 ± 2
	B	96	100	21 ± 2
1.2	A	89	100	20 ± 2
	B	83	100	20 ± 2
Control	A	88	96	21 ± 2
	B	93	98	21 ± 2
Solvent Control	A	85	100	21 ± 3
	B	91	92	20 ± 2

[a] $F=15.81$, $F_{0.05}(6,6) = 4.28$; $d' = 23.54$

[b] $F=9.0$, $F_{0.05}(6,6) = 4.28$; $d' = 1.69$

Table 11 -- Mean percentage hatch of fathead minnow (Pimephales promelas) eggs, and mean percent survival and mean total length of fry 30 days post hatch, during continuous exposure to replicate concentrations of elemental phosphorus in water.

Measured Phosphorus Concentration (ug/l)	Replicate	Hatch (%)	30 Days Post Hatch	
			Survival (%)	Total Length (mm)
6.8	A	76	100	15 ± 2[a]
	B	77	90	14 ± 2
2.5	A	79	98	16 ± 3[a]
	B	64	100	15 ± 2
1.5	A	83	100	15 ± 3[a]
	B	90	95	15 ± 2
0.7	A	77	98	16 ± 3
	B	89	100	16 ± 2
0.6	A	77	75	17 ± 2
	B	81	93	16 ± 2
Control	A	84	88	17 ± 3
	B	71	93	17 ± 2
Solvent Control	A	89	93	18 ± 2
	B	80	85	18 ± 3

[a] $F=20.33$, $F_{0.05}(6,6) = 4.28$; $d' = 1.10$

Table 12 -- Mean[a] percentage survival of Daphnia magna continuously exposed to elemental phosphorus over two generations.

Measured Phosphorus Concentration (ug/l)	n	% Survival					
		1st Generation			2nd Generation		
		Day/7	14	21	28	35	42
7.7	1	52[b]	29[c]	19[d]	-[e]	-	-
6.9	5	94	85	66	100	95	60
0.96	1	86	84	62	91	89	41
0.53	2	90	79	74	96	95	78
0.38	3	84	89	86	96	96	58
Control		90	84	68	90	88	80

[a]Based on the average of four replicate experimental units.

[b]F=4.26, F0.05 (5,15) = 2.90; d' = 2.90

[c]F=11.95, F0.05 (5,15) = 2.90; d' = 15.3

[d]F=16.61, F0.05 (5,15) = 2.90; d' = 12.9

[e]Insufficient animals produced during first generation exposure to continue the exposure through the second generation.

Table 13 -- Mean[a] number of young per parthenogenetic female *Daphnia magna* continuously exposed to elemental phosphorus over two generations.

Measured Phosphorus Concentration (ug/l)	Young/Parthenogenetic Female			
	1st Generation		2nd Generation	
Day/	14	21	35	42
8.7	0[b]	2[c]	-[d]	-
6.9	5	8	14	24
1.0	8	8	15	33
0.5	8	7	10	8
0.3	9	10	8	6
Controls	7	6	12	11

[a]Based on the average of four replicate experimental units.

[b]$F=10.53$, $F_{0.05}(5,15) = 2.90$; $d' = 3.5$

[c]$F=3.29$, $F_{0.05}(5,15) = 2.90$; $d' = 5.5$

[d]Insufficient animals produced during the first generation exposure to continue the exposure through the second generation.

Table 14 -- Mean[a] (S.D.)[b] percent survival of midge (Chironomus tentans) larvae continuously exposed to elemental phosphorus in water for 8 days.

Measured Phosphorus Concentration (ug/l)	Survival (%)
2.0	21 (12) [c]
0.95	34 (3) [c]
0.46	42 (16) [c]
0.26	52 (30) [c]
0.14	56 (17) [c]
Control	92 (10)

[a]Represents the mean of four replicate experimental units.

[b]Standard Deviation.

[c]F= 8.6, F0.05 (5,15) = 2.9; d' = 20.8

Table 15 -- Mean[a] (S.D.)[b] percent survival of midge (Chironomus tentans) larvae[c] and pupae[d], and percent survival and emergence of adults[d], and number of eggs produced/adult[d] during continuous exposure to elemental phosphorus in water.

Measured Phosphorus Conc. (ug/l)	n	Survival (%)			Emergence (%)	Eggs/Adult
		Larvae	Pupae	Adults		
0.067	2	74 (15)	96 (4)	28 (7)	100	15
0.023	2	66 (14)	99 (1)	26 (7)	100	23
0.012	2	54 (13)	96 (5)	26 (11)	84 (19)	15
0.005	2	57 (2)	96 (3)	36 (22)	92 (14)	7
Control		70 (5)	87 (24)	27 (21)	83 (34)	23

[a] Represents the mean of four replicate experimental units.

[b] Standard deviation.

[c] Determined after 14 days exposure.

[d] Determined after 28 days exposure

Table 16 -- Mean[a] (Standard Deviation) percent emergence and adult survival, and mean number of egg/adult for the first generation of midges (Chironomus tentans) inhabiting sediments containing elemental phosphorus.

Nominal Phosphorus Conc (ug/kg)[b]	Day 19		Day 23		
	Emergence (%)	Survival (%)	Emergence (%)	Survival (%)	Eggs/Adult
500	20 (4)[c]	66 (19)	36 (11)	44 (12)	0
200	18 (8)[c]	74 (19)	27 (14)	39 (27)	20
100	16 (7)[c]	95 (6)	25 (12)	67 (21)	9
20	21 (7)[c]	88 (11)	34 (7)	59 (19)	7
2	23 (6)[c]	90 (5)	32 (12)	56 (17)	7
Control	32 (3)	67 (14)	44 (8)	46 (8)	5

[a]Represents the mean of four replicate experimental units.

[b]Nominal concentrations at the initiation of the first generation midge exposure.

[c]$F=3.21$, $F_{0.05}(5,15) = 2.90$; $d' = 7.5$

Table 17 -- Mean[a] (Standard Deviation) percent emergence and adult survival, and mean number of eggs/adult for the second generation of midges inhabiting sediments containing elemental phosphorus.

Nominal Phosphorus[b] Concentrations (ug/kg)	Day 33 Emergence (%)	Day 33 Survival (%)	Eggs/Adult
500	7 (2)	64 (43)	0
200	26 (6)	54 (14)	28
100	10 (7)	72 (15)	4
70	6 (1)	74 (25)	6
2	17 (15)	53 (40)	30
Control	22 (11)	71 (30)	23

[a]Represents the mean of four replicate experimental units.

[b]Nominal concentrations at the initiation of the first gneration exposure.

Table 18 -- Measured water quality parameters during chronic exposure of fathead minnows (Pimephales promelas) to elemental phosphorus.

Parameter	Number of Samples	Mean and Standard Deviation	Range
Hardness (mg/l)	14	40 ± 2	26 - 42
Alkalinity (mg/l)	6	30 ± 8	21 - 42
Acidity (mg/l)	6	2.7 ± 1.8	1.0 - 5.7
DO (mg/l)	390	7.9 ± 0.6	6.4 - 9.1
pH	63	-	7.2 - 7.9

Table 19 -- Nominal and measured concentrations of elemental phosphorus in water during chronic exposure of fathead minnows (Pimephales promelas).

Nominal concentration (ug/l)	n	Measured concentration (ug/l)	
		Mean and Standard Deviation	Range
10.0	13	6.0 ± 1.9	3.7 - 9.9
5.0	19	3.4 ± 0.68	2.3 - 5.2
2.5	20	1.5 ± 0.49	0.95 - 3.0
1.2	20	0.71 ± 0.23	0.34 - 1.1
0.6	21	0.40 ± 0.11	0.19 - 0.60
Control	10	<0.13	<0.05 - <0.13

Table 20 -- Survival and growth of fathead minnow (Pimephales promelas) continuously exposed to elemental phosphorus in water.

Parameter	Mean measured phosphorus concentration (ug/l)													
	6.0		3.4		1.5		0.71		0.40		S. Control		Control	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Hatchability (%)	87	89	83	87	80	69	90	80	87	89	80	85	92	85
30 Days														
Survival (%)	85	100	100	100	93	88	100	95	98	98	93	83	95	95
Total length (mm)	13(2)[a,d]	13(2)	15(2)[d]	15(1)	17(2)[d]	17(2)	18(2)	18(2)	19(3)	18(2)	20(2)	20(2)	20(2)	19(2)
60 Days														
Survival (%)	55[e]	55	95	98	93	88	95	90	98	98	93	83	95	93
Total length (mm)	17(2)[f]	16(2)	20(3)[f]	19(3)	25(4)[f]	25(3)	28(4)	28(3)	28(4)	28(3)	30(4)	30(3)	29(3)	28(3)
150 Days														
Survival (%)[b]	0[g]	0	7[g]	20	67[g]	40	80	87	100	93	100	100	93	100
♂/♀	-	-	1[c]	3[c]	10[c]	6[c]	2/10	3/10	6/9	6/8	6/9	5/10	7/7	4/11

[a] Mean and standard deviation.

[b] Based on 15 fish per duplicate (after thinning on day 60).

[c] Total number of surviving fish - all fish immature (sex could not be determined).

[d] F = 167.73 F0.05 (6,6) = 4.28; d' = 1.01

[e] F = 24.53 F0.05 (6,6) = 4.28; d' = 12.04

[f] F = 368.15 F0.05 (6,6) = 4.28; d' = 1.42

[g] F = 32.08 F0.05 (6,6) = 4.28; d' = 32.21

AD-A061 785 EG AND G BIONOMICS WAREHAM MASS F/G 8/1
LABORATORY EVALUATION OF THE TOXICITY OF ELEMENTAL PHOSPHORUS (--ETC(U)
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Table 21 -- Size and reproductive potential of fathead minnow (Pimephales promelas) continuously exposed to phosphorus for 241 days.

Parameter	Mean measured phosphorus concentration (ug/l)													
	6.0		3.4		1.5		0.71		0.40		S. Control		Control	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
241 Days Survival	0	0	0	0	40	33	100	100	100	100	100	100	100	78
Total length (mm)														
males	-	-	-	-	35[a]	40[a]	55	59	62	55	61	63	61	61
females	-	-	-	-			43 [c]	45	48 [c]	48	48	50	50	52
Wet weight (g)														
males	-	-	-	-	0.32[a]	0.41[a]	1.63	2.25	2.68	1.92	3.39	3.00	3.02	3.35
females	-	-	-	-			0.68 [d]	0.81	0.98 [d]	1.05	1.13	1.29	1.21	1.37
Ratio ♂/♀	-	-	-	-	4[a]	2[a]	2/7	2/7	3/6	3/6	3/6	3/6	4/5	3/6[b]
Total spawns	0	0	0	0	0	0	3	23	13	20	27	23	27	29
Total eggs	-	-	-	-	-	-	152	1760	896	2989	2867	2097	2621	2520
Spawns/female	-	-	-	-	-	-	0.4	3.3	2.2	3.3	4.5	3.8	5.4	4.8
Eggs/female	-	-	-	-	-	-	22	251	149	498	478	350	524	420
Eggs/spawn	-	-	-	-	-	-	51	77	69	149	106	91	97	87

[a] Total number of surviving fish - all fish immature (sex could not be determined) and severely stunted in growth.

[b] Two females died on days 201 and 229 (middle of spawning period) and were counted as spawning females in egg production calculations.

[c] $F = 34.67$ $F_{0.5}(3,3) = 9.28$; $lsd(0.05) = 2.26$

[d] $F = 130.78$ $F_{0.05}(3,3) = 9.28$; $lsd(0.05) = 0.09$

Table 22 -- Effects on second generation eggs and fry of fathead minnow (Pimephales promelas) from continuous exposure to phosphorus.

Parameter	Mean measured phosphorus concentration (ug/l)													
	6.0		3.4		1.5		0.71		0.40		S. Control		Control	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Hatchability (%)														
Eggs from exposed parents (N)[a]	-	-	-	-	-	-	0(1)	3(10)	16(5)	0(10)	93(10)	90(10)	92(10)	94(12)
Eggs transferred to control (N)	-	-	-	-	-	-	0(1)	0(3)	15(3)	0(4)	-	-	-	-
Eggs transferred from control (N)	95(3)	95(3)	97(3)	98(3)	92(3)	93(3)	98(3)	92(3)	98(3)	98(3)	-	-	-	-
30 Days														
Survival (%)	80[b]	95[b]	100[b]	95[b]	93[b]	70[b]	95[b]	100[b]	93[b]	90[b]	98	98	90	98
Total length (mm)	16(2)[c,d]	16(1)	20(1)[d]	19(2)	19(2)[d]	20(3)	22(2)[d]	21(2)	22(3)[d]	20(2)	24(2)	24(2)	24(1)	25(2)
Mean wet weight (g)	0.03[e]	0.03	0.05[e]	0.05	0.05[e]	0.07	0.07[e]	0.07	0.10	0.08	0.13	0.11	0.11	0.14

[a] Number of egg groups exposed.

[b] Fry transferred from control.

[c] Mean and standard deviation.

[d] F = 27.39 F0.05 (6,6) = 4.28; d' = 2.96

[e] F = 14.56 F0.05 (6,6) = 4.28; d' = 4.95

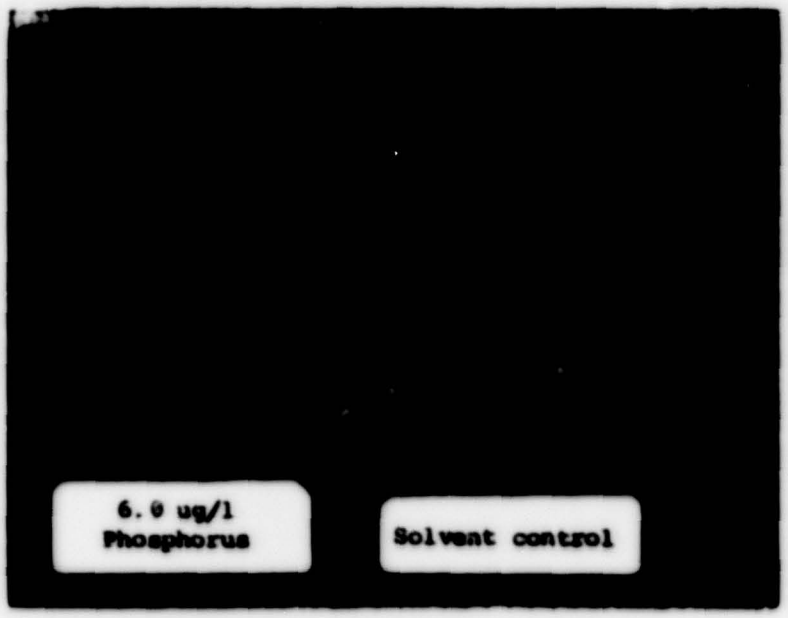

Figure 1. Representative sample of juvenile fathead minnows after 60 days post-hatch exposure to 6.0 μg/l phosphorus and diluent water containing an amount of dimethyl sulfoxide equal to that contained in the 6.0 μg/l phosphorus treated water. Each division is 2 mm.

Table 23 -- Concentrations of elemental phosphorus in water (ug/l) during exposure of channel catfish (*Ictalurus punctatus*) and fathead minnow (*Pimephales promelas*).

Species	Nominal Concentration of P4 (ug/l)	Measured P4 concentration in water (ug/l) Mean ± S.D.	Range	N
Ictalurus	2.0	1.8 ± 0.37	1.2-2.2	12
punctatus	0.20	0.18 ± 0.029	0.14-0.21	12
	Control	<0.017	-	7
Pimephales	2.0	2.2 ± 0.33	1.6-2.5	7
promelas	0.20	0.15 ± 0.046	0.074-0.23	17
	Control	<0.017	-	9

Table 24 -- Concentrations of phosphorus in channel catfish (ug/kg) during continuous exposure to 1.8 ug/l in water and during subsequent depuration in control water.

Period	Day	phosphorus concentration in tissue (ug/kg) Muscle	Liver	Remains
exposure	1	100	< 90	90
	2	190	<130	170
	7	130	<110	130
	14	430	< 22	140
	21	100	52	99
	28	120	100	140
	*33	46	< 86	41
	*34	180	120	110
	35	140	120	190
	*37	<13	<110	<13
	47	90	80	80
Depuration	2	< 4	< 40	10
	7	<11	<130	<14

*Mortalities occurred on this day and were used for analysis of phosphorus concentration.

Table 25 -- Concentrations of phosphorus in channel catfish (ug/kg) during continuous exposure to 0.18 ug/l in water and subsequent depuration in control water.

Period	Day	Phosphorus concentration in tissue ug/kg		
		Muscle	Liver	Remains
exposure	1	< 9	< 70	< 6
	2	<12	<130	<12
	7	<13	<140	<12
	14	12	< 36	< 7
	21	10	< 33	10
	28	5.7	< 27	< 5
	35	17	< 38	18
	47	11	< 72	16
Depuration	2	<4.1	< 52	5.4
	7	<14	< 15	< 17

Table 26 -- Concentrations of phosphorus in fathead minnows (ug/kg) during continuous exposure to 2.2 ug/l in water and subsequent depuration in control water.

Period	Day	phosphorus concentration in tissue (ug/kg) Muscle	Remains
exposure	1	<100	<180
	2	340	440
	7	180	230
	*12	34	360
	*13	67	200
	*14	41	54
	14	< 35	< 73
	*15	20	15
	*16	< 19	64
	*19	19	< 23
	*20	29	< 36
	*21	97	< 43
	21	< 46	< 80

* Mortalities occurred on this day and were used for analysis of phosphorus concentration.

Table 27 -- Concentrations of phosphorus in fathead minnows (ug/kg) during continuous exposure to 0.15 ug/l in water and subsequent depuration in control water.

Period	Day	phosphorus concentration in tissue ug/kg		
		Muscle	Remains	Ovaries
exposure	1	<40	<47	-
	2	<68	<97	-
	7	<64	<80	-
	14	<19	<19	-
	21	<24	<23	-
	28	<20	<24	-
	35	<23	<38	-
	47	<20	<29	-
	77	19	30	<31
Depuration	2	<21	<33	
	7	<30	<54	

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